TEST EXAMPLE 2

^{3}H-Iloprost Binding Inhibition Test to Human Platelet Membrane

Human platelet membrane of 100 μg protein was suspended in 200 μl of 50 mM Tris-HCl buffer (pH7.4) containing 10 mM $MgCl_2$, 1 mM EGTA and 5 nM ^{3}H-Iloprost and then incubated at 37° C. for 10 minutes with 5 μM non-labeled Iloprost or the compound (1 μM) of Example 64. The platelet membrane was collected on a glass filter and its radioactivity was measured after washing four times with 50 mM Tris-HCl buffer. The compound (1 μM) of Example 64 inhibited binding of ^{3}H-Iloprost by 85%.

TEST EXAMPLE 3

Increase in cAMP in Human Platelet

500 μl of washed human platelet suspension (2×10^8/ml) containing each concentration of the compound of Example 64 was incubated at 37° C. for 10 minutes and sonicated after adding aqueous 1 M perchloric acid solution. After the sonicated solution was centrifuged and the supernatant was neutralized with an aqueous 1 M potassium hydrogen carbonate solution, centrifuged again and the supernatant was recovered. The concentration of cAMP in the supernatant was determined by an ELISA method. As shown in Table 2, the amount of cAMP in platelets was increased by the compound of Example 64 in a concentration-dependent mannar.

TABLE 2

Increase in cAMP in human platelet.

Concentration of Example 64 (nM)	cAMP (pmol/10^8 platelet)
10	3.7
30	6.4
100	20.7
300	28.5
1000	39.4
3000	35.3

As is apparent from Test Examples 1 to 3, the compounds of the present invention inhibit platelet aggregation on the basis of their PGI_2 receptor antagonistic activity.

TEST EXAMPLE 4

Singl-Dose Toxicity Test in Mice

The compound of Example 42 was orally administered to mice (including three mice in a group) at a dose of 10, 30 or 100 mg/kg. As a result, there were no deaths.

The compound of Example 64 was orally administered to mice (including five mice in a group) at a dose of 300 mg/kg. As a result, there were no deaths.

TEST EXAMPLE 5

Ex Vivo Platelet Aggregation Inhibition Test in Monkeys

Method

The compound of Example 84 was orally administered to two cynomolgus monkeys (*Macaca fascicularis*, male, aged 3 to 5) at a dose of 0.3 or 1 mg/kg. Before the administration or 2, 4 and 8 hours after the administration, blood (each 4.5 ml) was collected using an injection cylinder containing aqueous 3.8% citric acid solution in the amount of 1/10 of the volume of blood to be collected. Blood containing citric acid solution was centrifuged at 200×g for 10 minutes and the upper layer was collected as PRP. Furthermore, the residual blood was centrifuged at 1500×g for 10 minutes and the supernatant was collected as PPP. 190 μl of PRP was added to a cuvette and incubated at 37° C. for one minute, and then 10 μl of ADP solution (5 to 30 μM) was added to induce platelet aggregation. The percentage of platelet aggregation was measured by an aggregometer (PM8C, Mebanix, Tokyo) using PPP as the blank and inhibition percentage of platelet aggregation was determined by comparing the percentage of aggregation before administration of the compound with the percentage of aggregation after administration of the compound according to the following equation. The results are shown in Table 3.

Inhibition percentage of Platelet Aggregation (%)=100−(The percentage of Aggregation after administration of compound)/(The percentage of Aggregation before administration of compound)×100

TABLE 3

Inhibition percentage of platelet aggregation in monkeys (%)

Time after administration	Dose of Example 84 (mg/kg)	
(h)	0.3	1
2	28	63
4	34	40
8	19	52

It is apparent that the compound of Example 84 inhibits platelet aggregation persistently in a dose-dependent manner after 2 to 8 hours have passed since the administration, and drug efficacy persists for a long time.

FORMULATION EXAMPLE 1

Tablets (tablets for internal use)

Formulation weighing 200 mg per tablet

Compound of Example 40	20 mg
Corn starch	88 mg
Crystalline cellulose	80 mg
Calcium Carboxymethylcellulose	10 mg
Light anhydrous silicic acid	1 mg
Magnesium stearate	1 mg

Mixed powders prepared according to the above formulation were compressed to give tablets for internal use.

FORMULATION EXAMPLE 2

Tablets (tablets for internal use)

Formulation weighting 120 mg per tablet

Compound of Example 51	1 mg
Lactose	60 mg